

Transmission of *Yersinia enterocolitica* 4:O3 from pig tonsils to edible offal

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Abstract: Distribution of pathogenic *Yersinia enterocolitica* was studied in a slaughterhouse in Southern Germany. A total of 120 pooled pig offal samples (tonsils, tongues, lungs, hearts, diaphragms, and livers) were from pluck sets hanging on racks and 20 pooled pig kidney samples were from containers. The highest isolation rate of pathogenic *Y. enterocolitica* 4:O3 was obtained from tonsils (85%) and the lowest from kidneys (15%). Altogether, 16 genotypes were obtained when the 122 isolates were characterised with PFGE using *NotI* enzyme. The high contamination rate of the tonsils and the indistinguishable genotypes obtained from the offal indicate that the tonsils contaminate the tongue, lungs, heart, diaphragm and liver when they are removed and hang on the hook together.

Keywords: *Yersinia enterocolitica*, slaughterhouse, PFGE

Introduction: The epidemiology of *Y. enterocolitica* infections is complex and poorly understood. Most cases of yersiniosis occur sporadically without an apparent source (Bottone, 1999). Specific antibodies against *Y. enterocolitica* O3/O9 have been reported to be common in German blood donors (Mäki-Ikola et al., 1997). This bacterium is considered to be a foodborne pathogen, even though pathogenic isolates have seldom been recovered from foods (de Boer, 1995). The aim of this study was to genotype pig tonsil and offal isolates to get more information on possible contamination routes in the slaughterhouse.

Materials and Methods: 140 pooled offal samples from fattening pigs in Southern Germany were studied. 120 samples from tonsils, tongues, lungs, hearts, diaphragms and livers were collected from 20 racks where they hang on. Twenty samples from kidneys were collected from 20 containers. The entire surface of

tonsils, tongues, lungs, hearts, diaphragms, livers and kidneys were swabbed such that one pooled offal sample contained the swabbed surfaces of 5 organs.

Y. enterocolitica was isolated using direct plating, overnight enrichment and selective enrichment in MRB and ITC. Colonies of typical 'bull's eye' appearance on CIN agar plates, which were urease-positive, were identified using API 20E. *Y. enterocolitica* isolates were bio- and serotyped. Calcium dependence and Congo red absorption, two plasmid-encoded virulence markers, were studied with Congo red-magnesium oxalate agar plates (CR-MOX). DNA isolation, restriction enzyme digestion and PFGE was performed according to Fredriksson-Ahomaa et al. (1999).

Results: Pig tonsils, tongues, lungs, hearts, diaphragms and livers, which hang together on a rack in the slaughterhouse, were highly contaminated with pathogenic *Y. enterocolitica* 4:O3. At least one positive sample was found from all 20 racks studied. The highest isolation rate was obtained from tonsils. Kidneys, which were not attached with the pluck set and did not hang on the rack, had the lowest isolation rate (Table 1).

Table 1. Prevalence of *Yersinia enterocolitica* 4:O3 on offal samples.

| Samples | No. of samples | No. of positive samples | Prevalence % |
|------------|-----------------|-------------------------|--------------|
| Tonsils | 20 ^a | 17 | 85 |
| Tongues | 20 ^a | 15 | 75 |
| Lungs | 20 ^a | 14 | 70 |
| Hearts | 20 ^a | 14 | 70 |
| Diaphragms | 20 ^a | 10 | 50 |
| Livers | 20 ^a | 5 | 25 |
| Kidneys | 20 ^b | 3 | 15 |

^a One sample contained swabbed surfaces of 5 organs from one rack.

^b One sample contained swabbed surfaces of 5 kidneys from one container.

122 *Y. enterocolitica* 4:O3 isolates were recovered from 78 offal samples. 102 of these isolates recovered from 72 samples were positive for the plasmid encoded virulence markers. Altogether, 16 genotypes were obtained when all the *Y. enterocolitica* 4:O3 isolates were characterised with *NotI* enzyme. Indistinguishable genotypes were recovered from tonsil and offal samples collected from 16 out of 17 racks. Three genotypes found in kidneys were also found in tonsils.

Discussion: The high contamination rate of tonsils, and the indistinguishable genotypes recovered from tonsils and offal lend further support to our hypothesis

that tonsils contaminate the offal when they hang together on a hook. Prevalence of *Y. enterocolitica* 4:O3 in pig tonsils and feces has shown to be 60 and 10%, respectively, in slaughter pigs in Southern Germany (Fredriksson-Ahomaa et al. 2000c). This indicates that tonsils are a more important contamination source than feces in the slaughterhouse. One reason for the low contamination rate of the kidneys may be that they were left in the carcass during evisceration and they did not hang together with the pluck sets on the racks. In Finland, the kidneys were highly contaminated with *Y. enterocolitica* 4:O3, however, they were removed along with the pluck set and hung together on a hook (Fredriksson-Ahomaa et al. 2000b). Three genotypes recovered from kidneys were also found in tonsils, which indicates that tools and workers may transfer yersinia from tonsils to other parts of the carcass.

Conclusions: As long as the head, with the tonsils and tongue, is not removed prior to evisceration and is not handled and inspected separately, it is difficult to control the spreading of *Y. enterocolitica* 4:O3 from tonsils to offal and the carcass.

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